90. (New) The method of claim 89, wherein the change of the level of the enzyme or mRNA is detected using a microplate reader.--

REMARKS

Status of the applications

Claims 1-21, and 43 are pending and stand rejected in the application. With entry of this amendment, claims 21 and 43 have been amended, and new claims 54-90 have been added. Support for the recitation of "living teleost" in claim 21 is found in the specification, e.g., on page 63, line 30 and page 69, line 2. The recitation of "a dye with affinity for dead cells" finds support in the specification, e.g., on page 60, lines 10-26. The recitation of teleosts over 12 hr old in claim 21 is supported by the disclosure in the specification that the teleost to be employed could be from 12 hours up to a few days after fertilization (see, e.g., page 68, lines 26-27; page 69, line 14; page 70, line 31; page 71, line 15; page 72, lines 29-31; and page 73, lines 24-25). The 12 hr period corresponds to the end of gastrulation (see, e.g., Col. 11, lines 39-40 of Yager et al., U.S. Patent No. 5,932,418, which was cited in the Office Action). After this period, specific tissues and organ start to develop within the teleost. Support for the amendment to claim 43 is found in the specification, e.g., on pages 81-83. New claims 54-74 respectively encompass the same subject matter as that of canceled claims 22-42, and new claims 75-84 respectively claim the same subject matter as canceled claims 44-53. Support for new claims 85, 87, and 89 is provided in the specification, e.g., on page 18, line 9; and page 21, line 28. The recitation of "microplate reader" in claims 86, 88, and 90 has support in the specification, e.g., on page 21, line 31. No new matter has been introduced by the claim amendment.

Claim amendments are for improved clarity or consistency of claim language unless otherwise noted and no claim amendment should be construed as an acquiescence in any ground of rejection. The following addresses issues raised by the Examiner in the Office Action.

Rejections under 35 U.S.C. 102

1. Rejection of claim 43 under 35 U.S.C. 102(b)

Claim 43 was rejected under 35 U.S.C. 102(b) as being anticipated by Zikria et al. (U.S. Patent No. 5,565,187). The Examiner says that Zikria et al. teach methods for studying capillary circulation using fish fry and tadpoles wherein the toxic agent is injected into the yolk sac and the capillary circulation is observed.

Claim 43 has been amended to recite that toxicity is determined by detecting a change in the level of an enzyme or RNA molecule in a specific organ or tissue responsive to administering an agent. Such detection is advantageous both in localizing the site of a toxic response, and in providing an indication of the nature and extent of the response (depending on the enzyme(s) or RNA(s) whose levels change, and the extent to which they change). The feasibility of such detection is illustrated by an example described on pages 81-84 of the specification in which the toxic effects of aspirin and deoxamethasone on the kidney and liver were assessed.

Zikria et al. discuss a process for analyzing toxicity in which a fish embryo is injected with a fluorescent dye, and fluorescent microscopy is used to monitor leakage of the dye from the systemic circulation of the embryo to surrounding tissues. Toxicity of a compound is indicated by increased leakage of the dye due to damage to surrounding tissue. This technique is relatively crude in that detects only a single type of response (i.e., gross tissue damage) rather than underlying molecular events, and does not distinguish between tissue damage that may result from injection of the dye as distinct from the that due to the toxic compound. Moreover, no data are presented to indicating that the method can detect differential responses in specific organs or tissues.

Zikria does not anticipate or render obvious claim 43, as amended, because Zikria does not disclose or suggest detection of changes in enzyme or mRNA levels in a specific tissue or organ as a measure of toxicity. Zikria discusses only one type of assay, namely a fluorescent dye leakage assay, and does not suggest any modifications or alternatives to this approach, or provide any rationale why such modifications might be desired. Zikria's rather crude form of assay also does not provide any indication that the more subtle and useful tissue-specific molecular changes recited in the present claims could be detected responsive to

administering a compound to a fish embryo. For these reasons, it is submitted that the rejection should be withdrawn.

2. Rejection of claim 21 under 35 U.S.C. 102(e)

Claim 21 was rejected under 35 U.S.C. 102(e) as being anticipated by Yager (U.S. Patent No. 5,932,418). The Examiner says that Yager et al. teach a fish embryo screening test for genotoxic agents, including those which cause apoptosis. Therefore, the Examiner concludes that claim 21 is anticipated by Yager et al.

Claim 21 has been amended to recite that teleosts are at least 12 hour old at the time of performing the assay, that the teleost is living at the time of performing the assay, and that the assay is performed by detecting a dye with affinity for dead cells in a specific tissue or organ of the teleost. The feasibility of such an approach is shown by the example in the paragraph bridging pp. 70-71 of the specification. Performing assays post 12 hr has the advantage of allowing detection of cell death in specific tissues (before the 12 hr stage, specific tissues are not yet discernable). Further, performing assays on live teleosts has the advantage that the same teleost can be used for a time course of measurements as described at pp. 70-71.

Yager et al. discuss treating teleost embryos with testing agents and then observing the embryos for damaged DNA . Such damage can be observed either as gross chromosomal aberrations or by DNA fragmentation (see col. 11, line 60 to col. 12, line 4). The latter is also an indicator, albeit nonspecific, of apoptosis. Such assays are performed on chemically fixed, flattened embryos (see Col 3). The chemical fixing results in a dead embryo. All observations were made before the end of the pregastrulation phase (Col. 2, line 52 to Col. 3, line 13; and Col. 11, lines 22-40) on undifferentiated cells. According to Yager, pre-gastrulation embryo are advantageous because of strong differential responses to toxicity at this stage (Col. 1, lines 41-44).

Claim 21 (as amended) is not anticipated or rendered obvious by Yager because Yager does not disclose or suggest performing assays post 12-hour of development, performing assays on living teleosts, or detecting cell death in specific organs or tissues. To the contrary, Yager indicates that performing assays at the pregastrulation stage is advantageous because of the strong differential response to genotoxicity at this stage (col. 1,

lines 41-44), thereby teaching away from the presently claimed invention. Further, Yager does not provide any indication of the means for performing assays in still living teleosts or of the feasibility of detecting cell death responses in specific organs or tissues. As such, it is submitted that the instant rejection should be withdrawn.

Rejections under 35 U.S.C. 103

Claims 1-13 were rejected under 35 U.S.C. 103(a) as being unpatentable over Stainier et al. (Trends in Cardiovas. Medicine, 4:207-212, 1994) in view of Driever et al. (Trends in Genetics, 10:152-159, 1994), Weinstein et al. (Nature Medicine, 1:1143-1147, 1995) and Ozato et al. (Cell Differentiation 19:237-244, 1986). Stainier, Driever and Weinstein are all cited as teaching use of zebrafish for mutagenesis and to analyze functions of certain genes, including genes affecting the vascular system in fish. Ozato is cited as teaching production of transgenic fish (presumably with respect to claim 12 that refers to a transgenic teleost). The Examiner says that it would have been obvious to modify the methods in the cited references to achieve the claimed methods (1) because Stainier et al. and Driever et al teach that zebrafish is an attractive system to screen for mutations and to understand development of the cardiovascular system and organogenesis, and (2) because the Stainier abstract states that "our further understanding of the development of the cardiovascular system is important not only because of the high incidence and familial inheritance of congenital abnormalities, but also because it should lead to novel differentiation-based strategies for the analysis and therapy of the diseased state." This rejection is respectfully traversed.

The three primary references, Stainier, Driever and Weinstein, are all principally directed to the use of zebrafish for performing mutagenesis studies as a means of understanding gene function. The general goal of such analyses is most succinctly stated in the title and abstract of the Weinstein paper namely, such mutagenesis provides a "genetic tool" that "enhance our understanding of vertebrate development." However, none of these references provide any disclosure of using zebrafish to screen drugs, whether for angiogenic activity, as claimed, or otherwise.

The first source of motivation cited by the Examiner for modifying the teaching of the above references (attractiveness of zebrafish to screen mutations and understanding

development of the cardiovascular system and organogenesis) in fact suggests performing additional work in the same manner as described in the references. That is, to conduct mutagenesis as a means to understanding the function of mutagenized genes, and thereby the mechanism of cardiovascular systems and organogenesis. Such is entirely consistent with the visions for future research discussed at the end of the Stainier reference:

> The approach we are taking is to systematically screen for mutations affecting the development of cardiovascular form and function....This should help us lean more about cardiovascular development through the isolation of specific mutations and also through the use of more targeted genetic manipulations of the embryo, including transgenesis. (Stainier at p. 211, third column).

Likewise, Driever's future plans of using zebrafish to mutate and determine functional roles of genes discovered in the Human Genome Project (see p. 158 of Driever) suggest additional mutagenesis and functional studies as discussed in all three primary references. Accordingly, the first source of motivation asserted by the Examiner is not a motivation to modify the teaching of the cited references, particularly in the manner claimed, but to continue doing the type of experiments discussed in the references.

The second source of motivation asserted by the Examiner (Stainier's reference to differentiation based therapies) also does not necessarily point to the claimed methods. Stainier does not explicitly say how he envisages that novel-differentiation based therapies will be developed, and the Examiner simply assumes that this would be by the claimed methods. Applicants respectfully suggest that this assumption is colored by the hindsight of reading applicant's disclosure, and that based on the text of the Stainier reference itself, Stainier probably had a different idea in mind. That is, Stainier proposed to use mutagenesis of zebrafish as a means to understand the mechanism of vascular differentiation in zebrafish, and then to use this knowledge to design strategies of therapeutic intervention suitable for use in vertebrates. Such is consistent with Stainier's reference to differentiation based therapies in the context of the "high incidence of and familial inheritance of congenital abnormalities" (see abstract) and with Stainier's plans for future work to "screen for mutations affecting the development of cardiovascular form and function" (p. 211, third column). Thus, the goal of

producing differentiation-based therapies can logically be pursued without departing from the teaching of the cited references to conduct mutagenesis and analyze function.

To be cognizable, motivation must have sufficient "force" to "impel persons skilled in the art to do what applicant has done." *Ex parte Levengood,* 28 USPQ2d 1300, 1302 (BPAI 1993). Further, when all the facts have been considered, if the evidence is in "equipoise," an inventor is "entitled to a patent." *In re Oetiker,* 24 USPQ2d 1443, 1447 (Fed. Cir. 1992) (Plager, J., concurring). Here, neither source of motivation advanced by the Examiner would have impelled the artisan to do what the present inventors have done. As discussed above, it seems more likely that these forms of motivation would result in the reader performing additional mutagenesis and functional characterization experiments of the type described in the cited references. Even if the evidence were in equipoise as to whether the asserted motivation would lead one to the claimed invention or would lead one to continue in the same manner as described by the references (which is denied), then applicants would be entitled to a patent.

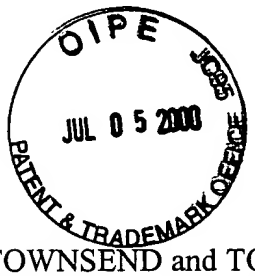
For all of these reasons, withdrawal of the rejection is respectfully requested.

Dependent claims are submitted to be nonobvious for at least the same reasons as claim 1.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400, ext. 5218.



Respectfully submitted,

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